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Topoisomerase I and II inhibitors: chemical structure, mechanisms of action and role in cancer chemotherapy

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The review summarizes and analyzes recent published data on topoisomerase I and II inhibitors as potential antitumour agents. Functions and the mechanism of action of topoisomerases are considered. The molecular mechanism of interactions between low-molecular-weight compounds and these proteins is discussed. Topoisomerase inhibitors belonging to different classes of chemical compounds are systematically covered. Assays for the inhibition of topoisomerases and the possibilities of using the computer-aided modelling for the rational design of novel drugs for cancer chemotherapy are presented.
The bibliography includes 127 references.

Contents

I. Introduction

The improvement of the efficacy of the treatment of cancer patients is the biggest social problem in the world in general and in Russia in particular. One of approaches to solving this problem is based on the design of new compounds acting on targets essential for cancer cell survival. Current treatment regimens for cancer patients involve drugs which cause cancer cell death and have proved to be effective therapeutic agents. This strategy has gained acceptance during a half century of development of cancer chemotherapy and undoubtedly provides an important role of drug therapy in the complex treatment of patients. The identification of particular intracellular targets for chemotherapeutic agents and the elucidation of the molecular mechanisms of their action became possible only in recent years due to the progress in molecular biology methods.

Rapidly growing knowledge on the molecular mechanisms of carcinogenesis suggested a new paradigm for the design of new effective antitumour agents. Thus, it is supposed that a compound which acts on a particular target in the cancer cell with minimal damage to surrounding healthy cells is therapeutically advantageous. It is reasonable to expect that oncoproteins responsible for the distinguishing features of cancer cells, such as morphological transformations, high cell proliferation, ability to survive

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under adverse external factors and so on, can serve as targets for cancer therapy. The development of methods of computational modelling of biological systems and high-throughput screening of vast chemical libraries offer the possibility of the targeted action not only on a particular oncoprotein but also on its environment, *i.e.*, on (macro)-molecules, interactions with which are necessary for the functioning of such proteins. The present review considers the problem of the design of antitumour agents based on inhibitors of topoisomerases — enzymes essential for the maintenance of the transformed phenotype of cancer cells.[1] These inhibitors were found in numerous classes, which makes it possible to reveal the structure–function relationship for the optimization of known drugs (see, for instance, the official site of the Group of companies RLS (Russian Drug Directory) www.rlsnet.ru/fg_index_id_272.htm) and the design of new agents.

II. Functions and mechanism of action of topoisomerases

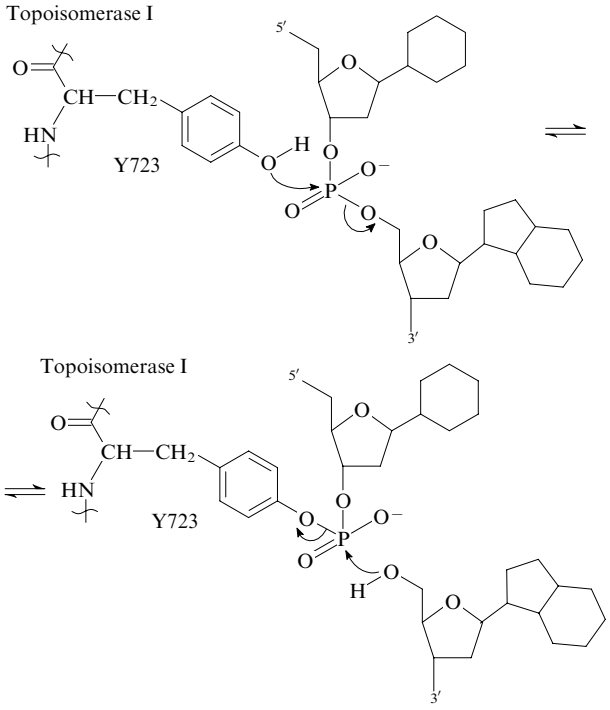
DNA-modifying enzymes — topoisomerases, polymerases, various transferases and so on — accomplish such essential processes in living cells as nucleic acid transcription, replication, mitosis and repair. It is reasonable to consider enzymes essential for tumour cell biology as targets for cancer chemotherapy. Actually, many low-molecular-weight chemical compounds can penetrate the cell nucleus and interact directly with DNA or inlfuence DNA-dependent enzymes. These interactions disrupt the structure and functions of DNA. Thus, they change the local conformation of the macromolecule, induce the strand cleavage and impair matrix syntheses. In response to DNA damage, the cell cycle is arrested and the cell viability decreases, resulting in the therapeutic effect, *e.g.*, the proliferation arrest and(or) tumour death.

The above considerations lie behind the search for new inhibitors of DNA-modifying enzymes, in particular topoisomerases, which catalyze topological rearrangements in DNA.[1] Topoisomerases introduce single- (topoisomerase I) or double-strand (topoisomerase II) breaks followed by the rejoining of DNA ends and the repair of DNA damage, resulting in the lability essential for conformational changes in DNA in the matrix DNA synthesis and for providing the mobility of chromosomes in mitosis.[2] Topoisomerases are known to be intracellular targets for chemotherapeutic agents, since topoisomerase inhibitors prevent these processes.

The impairment of DNA repair and the DNA damage accumulation are important mechanisms leading to the cell death.[1] Topoisomerase I breaks the phosphodiester bond in the DNA polynucleotide strand and covalently binds to one of the ends of the break. The active-site tyrosine of topoisomerases is essential for the interaction with DNA. In this case, the cleavage–ligation of the phosphodiester bond does not require external energy sources because the reaction products are chemically identical to the starting compounds. On the contrary, topoisomerase II and DNA gyrase use ATP energy to perform conformational transitions in the enzyme–substrate complex.[1–3]

The molecular mechanisms of the topoisomerase-mediated DNA topological transformations are considered in detail in the review by Yakubovskaya and Gabibov.[2] In the mentioned review, main conformational rearrangements of the duplex, such as knotting, catenation, linking and so on, are analyzed. On the whole, the catalytic mechanism of topoisomerases I and II can be represented as follows. The topoisomerase-catalyzed DNA relaxation is initiated by the nucleophilic attack of the OH group of the active-site tyrosine on the internucleoside phosphate group to form a covalent phosphotyrosine bond between one end of the break of the DNA strand (strands) and the enzyme, the other end of the DNA strand remaining free. Another DNA strand (strands) passes through the transient break in the DNA, and the attack of the hydroxyl group of the free end of the strand on the phosphotyrosine bond results in the religation of the DNA strand (strands). After the ligation of the DNA break, the enzyme can start a new catalytic cycle or be released from the relaxed DNA.[2,3] The mechanism of the topoisomerase I-mediated cleavage–ligation of the phosphodiester bond in DNA is shown in Scheme 1 (nucleosides are represented by contours of the rings).[4]

Scheme 1



Topoisomerase I is highly substrate selective. In particular, the primary structure and variability of DNA conformations play an important role. The DNA helix parameters depend on the conformations of sugar residues, phosphate groups and nitrogenous bases. In turn, this structural diversity can take place at the level of single nucleotides and short DNA fragments, and also at at longer helix regions having higher-order effects on the conformation, such as bending, kinking, looping and so on. Topoisomerase I interacts with ten nucleotide pairs and forms a specific hydrogen bond with the T(−1) base of the DNA; other enzyme–DNA contacts are less specific. Apparently, the T(−1) base of the broken DNA strand is essential for the initial selection of thymine-containing regions by the enzyme. In most cases, the break site contains the T(−1)–Pu(+1) sequence (Pu is a purine base).[5]

Topoisomerase II is required for the condensation and segregation of mitotic chromosomes. Hence, cells in which

this enzyme is inhibited are non-viable. Human cells contain two isoforms of this enzyme (IIα and IIβ). The activity of topoisomerase IIα increases in proliferating cells, the highest activity being observed in the S phase of the cell cycle.[6] The relationship between the S phase of the cell cycle and the activity of topoisomerases is evidenced by the termination of the replication after the pharmacological inhibition of these enzymes.[7] There are indications that the oncoproteins Ras, Myb and p53 interact with the topoisomerase IIα gene promoter region. Like topoisomerase I, topoisomerase II forms an intermediate complex with a DNA region and introduces a double-strand break, which is ligated by the enzyme, resulting in DNA repair and conformational changes.[8,9]

Main classes and characteristic structural features of low-molecular-weight topoisomerase inhibitors are considered below.

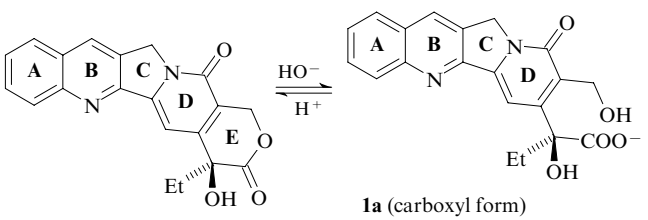
III. Topoisomerase inhibitors

1. Topo-poisons and topo-suppressors

Topoisomerase inhibitors can be divided into two groups, which differ in the mechanism of inhibition of the enzyme catalytic activity. Compounds belonging to one group (prototypes are the natural alkaloid camptothecin and its derivatives for topoisomerase I and etoposide for topoisomerase II) specifically inhibit the enzyme catalytic activity by stabilizing covalent DNA–topoisomerase complexes.[1,10,11] These compounds are referred to as specific inhibitors or topoisomerase poisons.[12–14] In the absence of inhibitors, covalent DNA–enzyme complexes are highly labile and dissociate to form the active enzyme and religated DNA. Topoisomerase poisons, in particular camptothecin, interfere with the religation step of the catalytic reaction, resulting in the DNA damage accumulation. Unrepaired DNA damages are prominent triggers for cell cycle arrest and activation of programmed cell death cascades (apoptotic cascades).[15] The paradigm of steric inhibition of the topoisomerase activity, *i.e.*, the inhibition *via* stabilization of the nucleotide strand (strands) in intermediate states due to the steric hindrance to the DNA–enzyme interaction (interfacial inhibition), substantiates the mechanism of action of topoisomerase poisons and can be employed to devise a strategy for the search for new inhibitors of this class.[16–18]

The history of the discovery of enzymes that catalyze topological rearrangements of DNA is related to investigations of the mechanism of action of the plant alkaloid camptothecin (**1a**), which is a specific inhibitor of eukaryotic topoisomerase I (Scheme 2). Camptothecin was isolated from the stem wood of the plant *Camptotheca acuminata* native to Tibet.[1]

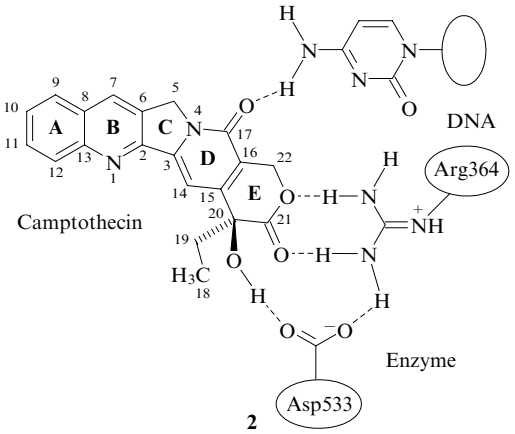
Scheme 2



1a (lactone form) **1a** (carboxyl form)

Camptothecin and its derivatives interact with both the enzyme and DNA, thus stabilizing the covalent DNA–topoisomerase I complex, increasing its lifetime and preventing DNA repair.[1,10,14] Camptothecin has a pentacyclic ring system, which includes the pyrrolo[3,4-*b*]quinoline moiety and the conjugated 2-pyridone ring. The possibility of the formation of two forms — lactone and carboxyl (see Scheme 2) — is responsible for the pH dependence of the pharmacological activity of camptothecin.[12] The lactone form of camptothecin is active and plays an important role in the formation of the ternary complex of camptothecin with DNA and topoisomerase I (Structure **2**).[4,12,19] Camptothecin poisons topoisomerase I, thus blocking the religation and promoting the formation of unrepaired DNA breaks.[1,14]

Structure 2

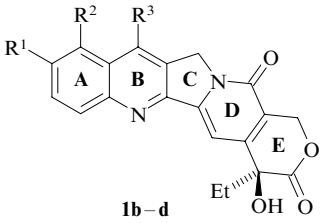


2

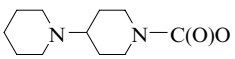
The α-hydroxylactone ring (**E** ring) containing one chiral centre at position 20 with the *S* configuration is the most important moiety in the structure of camptothecin. This ring interacts with the enzyme at three sites (see Structure **2**). The hydroxy group at position 20 forms a hydrogen bond with the side chain of Asp533 of the enzyme. The *S* configuration of the chiral carbon atom is of principal importance, because the *R* configuration is inactive. The lactone ring is linked by two hydrogen bonds to the guanidine group of Arg364. The **D** ring interacts with cytosine on the uncleaved DNA strand and stabilizes the DNA–topoisomerase I complex *via* hydrogen bonding between the carbonyl group at position 17 of the **D** ring and the pyrimidine amino group of the cytosine ring.[4] The cytotoxicity of camptothecin is due also to the conversion of DNA single-strand breaks to double-strand breaks occurring in the S phase, when the complexes formed by topoisomerase I, camptothecin and DNA create impediments to the movement of the replication fork.[1]

Camptothecin is of little clinical use because of high systemic toxicity and very low solubility in aqueous media. Hence, along with investigations of the effect of this compound, its synthetic analogues with higher hydrophilicity and lower side effects are searched for.[10,12,20] Two water-soluble camptothecin derivatives — topotecan (**1b**) (Hycamtin®, GlaxoSmithKline) and irinotecan (**1c**) (CPT-11, Camptosar®, Yakult Honsha KK) — are used in chemotherapy of ovarian and colorectal cancer. Other derivatives are in clinical trials.[10,12,20,21]

Structures 1b–d



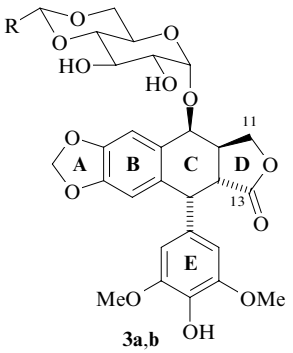
1b–d

Compound	R^1	R^2	R^3
Topotecan (**1b**)	OH	CH_2NMe_2	H
Irinotecan (**1c**) (CPT-11)		H	Et
SN-38 (**1d**)	OH	H	Et

Compounds of the camptothecin family, in particular topotecan, can cause DNA single-strand breaks in the absence of the enzyme not only at low but also at physiological ionic strength of the solution and can introduce double-strand breaks.[19] In solution, topotecan molecules form dimers and bind to DNA mainly as dimers [19, 22, 23] at its minor groove.[24, 25] The active metabolite of irinotecan **1d** (SN-38), as well as its derivatives, such as 9-aminocamptothecin, 9-nitrocamptothecin, *etc.*, exhibit high antitumour activity.[1, 15]

Etoposide (VP-16, **3a**), which a specific topoisomerase II inhibitor,[26] is a semisynthetic derivative of podophyllotoxin isolated from the roots of the plant *Podophyllum peltatum*. Etoposide is used in chemotherapy of ovarian cancer, gastric cancer, Hodgkin's lymphoma, acute leukemia, soft-tissue sarcomas and neuroblastoma. This compound is included in the list of vital and essential pharmaceuticals and strategically important drugs approved by the Government of the Russian Federation.

Structures 3



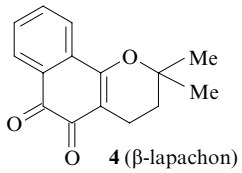
3a,**b**

R = Me (**a**, etoposide),
2-Th (**b**, teniposide)
(Th is thienyl)

Etoposide binds to topoisomerase II and inhibits DNA ligation, resulting in the accumulation of DNA breaks, the blockage of DNA replication and transcription, cell cycle arrest and, finally, the cell death.[26] Chemical groups of the **A**, **B** and **E** rings of etoposide are involved in interactions between topoisomerase IIα and etoposide. Although the **D** ring of etoposide does not participate in the interaction with topoisomerase II in the binary complex, the changes in this ring (the migration of the carbonyl group of lactone from position 13 to position 11, the opening of the **D** ring) increase the ability of etoposide to form a complex with topoisomerase II. This also leads to an increase in the ability of etoposide to form a ternary covalent complex and to changes of the binding sites.[27]

In addition to topo-poisons, a series of compounds which can impair the functions of topoisomerases without forming a covalent complex with the enzyme and DNA were found. This phenomenon is referred to as nonspecific inhibition of topoisomerases, and the corresponding inhibitors are called nonspecific inhibitors or suppressors.[4] For example, the natural compound β-lapachon (**4**) can suppress the topoisomerase I activity without forming a covalent complex.[28]

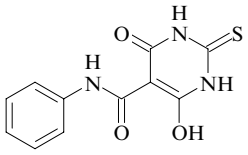
Structure 4



4 (β-lapachon)

The effective topoisomerase II inhibitor — merbaron (**5**) — is 5-carboxy-2-thiobarbituric acid anilide (phenylamide). It is supposed that merbaron can compete with topoisomerase II for the DNA binding site. The action of merbaron on cells is accompanied by DNA breaks and impairment of the cell cycle.[29] About 700 analogues and derivatives of barbituric acid were studied at the National Cancer Institute (USA) on a cancer cell line panel, but only merbaron proved to be active.[26]

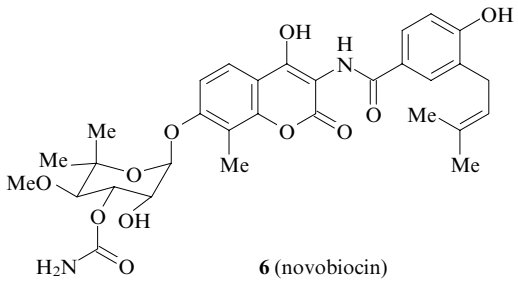
Structure 5



5 (merbaron)

The antibiotic novobiocin (**6**) from actinomycetes was found to be able to inhibit conformational rearrangements of DNA. This coumarin derivative is used in the treatment of bacterial infections. Novobiocin inhibits bacterial DNA gyrase B and mammalian topoisomerase II.[30] This agent can increase the cytotoxicity of etoposide and teniposide (**3b**) on certain cancer cell lines.[31]

Structure 6



6 (novobiocin)

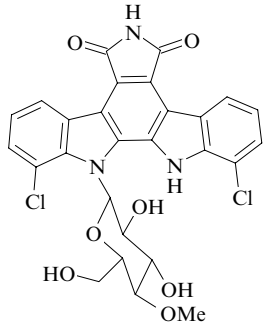
2. Non-camptothecin specific topoisomerase I inhibitors

The growing number of topoisomerase I inhibitors belonging to different classes of chemical compounds, as well as compounds with dual inhibitory activity against both topoisomerase I and topoisomerase II,[12, 32, 33] need to be systematized. It seems reasonable to classify inhibitors of different chemical nature into groups characterized by a

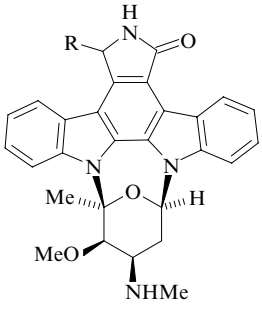
particular effect on the catalytic activity of these enzymes. Since camptothecin was studied in detail as a topoisomerase I inhibitor, inhibitors are classified into camptothecin-type inhibitors (camptothecin, its analogues and derivatives) and non-camptothecin derivatives.[11] Pommier and co-workers [34] distinguish three main classes of compounds capable of binding to topoisomerase I and DNA *via* the formation of a stable ternary covalent complex: camptothecins (see above), indolocarbazoles and indenoisoquinolines. The number of such compounds steadily increases due to the design of new heterocyclic DNA-binding ligands acting as topoisomerase poisons.[35–37]

Indolocarbazoles belong to one of the most well-known groups of topoisomerase I poisons. The natural antibiotic rebeccamycin (**7**), which is a glycosyl-substituted indolocarbazole, is a promising antitumour agent.[38, 39] The presence of a sugar residue in the antibiotic molecule leads to the enhancement of the inhibitory activity against topoisomerase I. Synthetic analogues of rebeccamycin, which have different modifications of the carbohydrate residue and differ in the position and nature of substituents in the chromophore, can influence the enzyme activity.[40, 41]

Structures 7, 8



7 (rebeccamycin)



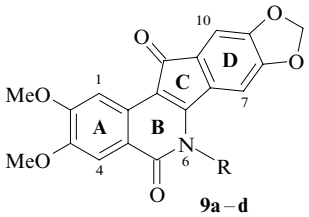
8a,**b**

R = H (**a**, staurosporine, OH (**b**, UCN-01)

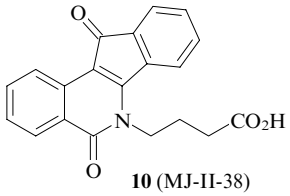
The natural indolocarbazole staurosporine (**8a**) is not a topoisomerase poison. However, certain synthetic derivatives of this compound form covalent complexes with DNA and topoisomerase I.[42] It should be noted that, in addition to topoisomerases, protein kinases are also targets for certain indolocarbazole derivatives.[41, 43] For instance, the compound UCN-01 (**8b**) exhibiting inhibitory activity against topoisomerase I was clinically tested as an antitumour agent, which also inhibits protein kinase C.[44]

Indenoisoquinolines belong to a large group of specific topoisomerase I inhibitors. The synthetic indenoisoquinoline NSC314622 (**9a**) was described for the first time in 1978.[45] More recently, it was found that compounds of this class (NSC314622 and MJ-III-65, **9b**) exhibit antitumour activity by inhibiting topoisomerase I.[11] Unlike camptothecin, indenoisoquinolines are chemically stable. The investigation of the complex formation of NSC314622 with DNA in the presence of topoisomerase I showed that the binding site of this inhibitor differs from the camptothecin-binding site.[35, 46] In addition, DNA breaks caused by the complexation of NSC314622 with DNA and topoisomerase I proved to be more stable than those in the case of camptothecin. This is essential for cancer chemotherapy since the rapid reversible complexation with camptothecin and low solubility of camptothecin require higher doses of the agent.[47] Particular attention is given to the evaluation of the antitumour activity of new derivatives on animal models and X-ray diffraction studies of complexes of the ligands under consideration (for example, MJ-II-38, **10**) with DNA and topoisomerase I.[47] Some indenoisoquinolines, in particular indotecan (NSC724998, **9c**) and indimitecan (NSC725776, **9d**), are currently under clinical trials.[47, 48]

Structures 9, 10



9a–d

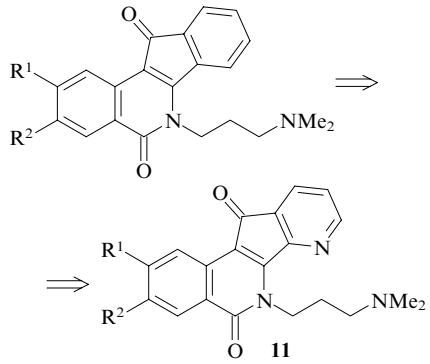


10 (MJ-II-38)

R = Me (**a**, NSC314622); $(CH_2)_3NH(CH_2)_2OH$ (**b**, MJ-III-65), $(CH_2)_3N(CH_2CH_2)_2O$ [**c**, NSC724998 (LMP400)], $(CH_2)_3Im$ [**d**, NSC725776 (LMP776)] (Im is imidazol-1-yl)

Investigations of the structure–function relationship in a series of new indenoisoquinoline derivatives, which can inhibit topoisomerase I and exhibit antitumour activity, are of great interest.[47–50] It was found that the presence of the dioxole ring conjugated to the **D** ring, as well as the presence of various substituents at the N(6) atom, have a substantial effect on the inhibition of topoisomerase I (see structures **9**).[49] The introduction of the nitrogen atom into the aromatic system of indenoisoquinolines (structures **11**, Scheme 3) increases the solubility of the corresponding derivatives in water without decreasing their anti-enzymatic and cytotoxic activities.[48]

Scheme 3



11: $R^1 = R^2 = H$, OMe; $R^1 = H$, $R^2 = NO_2$

The structures of the complexes formed by topoisomerase I with DNA and camptothecin and with such topoisomerase poisons as indenoisoquinolines and indolocarbazoles were established by X-ray crystallography. The planar structures of the heterocyclic moieties of the inhibitors allow them to intercalate into DNA in the vicinity of the break introduced by the enzyme. These compounds have a free electron pair involved in the hydrogen bonding with the residue Arg364 at which mutations are associated with the resistance to these classes of drugs. The intercalation of inhibitors is accompanied by the formation of contacts with Asn352 and Glu356. The side chains of these residues adopt alternative conformations optimal for the binding of the inhibitor. These data provide an explan-

ation of how structurally different molecules can stabilize covalent DNA–topoisomerase I complexes, which may be essential for the design of new antitumour agents.[14, 34, 35]

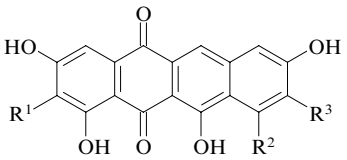
The topoisomerase activity substantially depends on various DNA damages (apurinic and apyrimidinic sites, breaks upon UV radiation and so on). If these regions are in the vicinity of the DNA-binding sites of the enzyme, the function of the enzyme is stimulated due probably to an increased affinity of topoisomerases for the damaged DNA.[51–53] Both the local DNA conformation and the structure of the macromolecule (integrity of the DNA strand, sequence specificity) are essential for the therapeutic effect because DNA-binding ligands can predominantly interact with particular DNA forms (including complex forms, such as guanine quadruplexes), as well as with free (unpaired) regions.[54–57] It seems reasonable to combine methods that are used to introduce DNA breaks in cancer cells (for example, ionizing radiation) with agents having affinity for the damaged strand (strands) to enhance the therapeutic effect.

3. Topoisomerase I and II inhibitors (dual inhibitors)

In the large family of non-camptothecin compounds, inhibitors capable of decreasing the activity of both topoisomerase I and topoisomerase II occupy a special place.[12]

The antibiotic saintopin (**12a**)[58] with a naphthacenedione structure is a representative of such compounds.

Structures 12



12a–d

Compound **12**	R^1	R^2	R^3
Saintopin (**a**)	H	OH	H
Saintopin E (**b**)	H	OH	Ac
UCE 1022 (**c**)	H	OSO_2OH	H
UCE 6 (**d**)	Me	$CH_2C(O)CH_2CHMeOH$	H

Saintopin induces the formation of covalent complexes of DNA with topoisomerases I and II in a cell-free system.[58] At low concentrations ($< 10^{-6}$ mol litre^{-1}), the ability of saintopin derivatives to cleave DNA increases in the series:

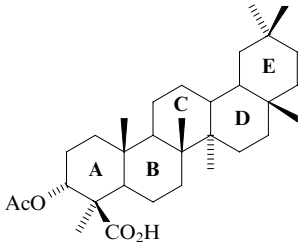
UCE 6 (**12d**) > saintopin (**12a**) > saintopin E (**12b**) >

> UCE 1022 (**12c**)

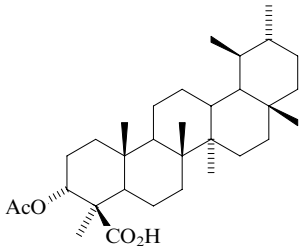
At higher concentrations, saintopin E can inhibit the formation of DNA–topoisomerase I complexes through a direct interaction with the enzyme, thus preventing its binding to the DNA.[58, 59]

The dual inhibitors — acetyl-boswellic acids **13a**,**b** — influence the activity of topoisomerases I and II without stabilization of covalent complexes and intercalation into DNA. Due to high enzyme-binding constants, their derivatives interact directly with topoisomerases I and II and reduce their activity.[60]

Structures 13



13a (3-*O*-acetyl-α-boswellic acid)

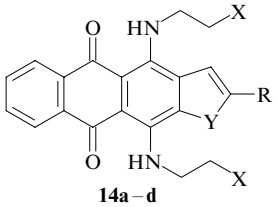


13b (3-*O*-acetyl-β-boswellic acid)

The number of compounds exhibiting inhibitory activity against topoisomerases I and II increases with each year.[61] Some of them, for example, evodiamine, which was isolated from the fruits of the plant *Evodia rutaecarpa* and is used in traditional Chinese medicine, are natural alkaloids.[62] Other compounds are synthetic products.[63]

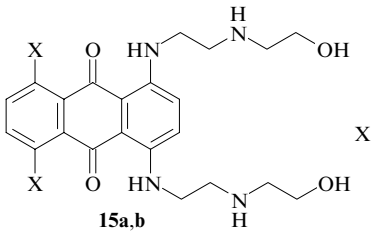
Shchekotikhin and co-workers[64–67] developed a new class of chemical compounds, *viz*., heteroareneanthraquinones **14a–d**, which possess antitumour activity and impair the functions of topoisomerases I and II. These compounds are structural analogues of the chemotherapy medications mitoxantrone and ametantrone(**15a**,**b**).

Structures 14, 15



14a–d

Y = O, R = Me: X = NHMe (**a**), NHC(=NH)NH_2 (**b**);
R = H, X = NHMe:
Y = NH (**c**), S (**d**)



15a,**b**

X = OH (**a**, mitoxantrone), H (**b**, ametantrone)

It was established that there is the relationship between the cytotoxicity of this class of compounds and the inhibition of topoisomerase I. It was shown that these properties are determined by the structural features of the inhibitor, such as the nature of the fused heterocycle, the nature and length of substituents and the charge distribution. The highest antiproliferative activity against human cancer cell lines (colon adenocarcinoma HCT116, leukemia K562) was found for heteroareneanthraquinones **14a**,**b** containing the furan moiety. This correlates with the suppression of topoisomerase I activity at submicromolar and low micromolecular concentrations. The guanidine derivative containing the furan moiety proved to be a more effective topoisomerase I inhibitor than its methylamine analogue.[67] It is important to determine whether heteroareneanthraquinones inhibit topoisomerase II as well.

4. Inhibitors active mainly against topoisomerase II

There are three main modes of binding of low-molecular-weight compounds to double-stranded DNA: external electrostatic binding, intercalation and binding in one of the grooves of B-form DNA.[68,69] Actinomycin D (**16**), aclarubicin (**17**) and doxorubicin (**18**) bind to the DNA molecule in such a way that the planar aromatic part of the molecule intercalates into DNA, and the bulky functional groups reside in the minor groove thus stabilizing the complex. These compounds with a mixed mode of binding to DNA also can induce the formation of ternary covalent complexes.[32,70,71]

The antitumour anthracycline antibiotic aclarubicin (**17**, or aclacinomycin A) is used in the therapy of acute leukemia.[26] This antibiotic is a strong intercalator and topoisomerase II inhibitor.[9] At particular concentrations, aclarubicin can suppress the function of topoisomerase I as well.[72] High concentrations of aclarubicin stimulate the formation of covalent DNA–topoisomerase I complexes. At low concentrations, this antibiotic prevents the binding of topoisomerase I to DNA acting as a non-specific enzyme suppressor.[73]

The anthracycline antibiotic doxorubicin (**18**, or adriamycin) is a well-known topoisomerase II inhibitor.[74,75] However, in some publications, this compound and its certain derivatives were characterized as topoisomerase I inhibitors.[70,76,77] This antibiotic is clinically used for the treatment of acute lymphoblastic leukemia, non-Hodgkin's lymphomas, breast cancer, small-cell lung cancer, pancreatic cancer, soft-tissue sarcomas and so on.

Mitoxantrone (**15a**) is as effective topoisomerase II inhibitor as adriamycin, but these agents differ in the therapeutic action spectrum. Mitoxantrone is used for the treatment of acute leukemia, lymphomas and prostate cancer, whereas adriamycin has a broader spectrum of clinical applications, from hemoblastoses to solid tumours of different tissue origins. The clinical use of adriamycin is limited by its high systemic toxicity manifested in cardiomyopathy and cytopenia. The mechanisms of these phenomena are associated with intercalation-induced DNA damage of non-cancer cells, the formation of active oxygen forms, release of iron and so on.[78]

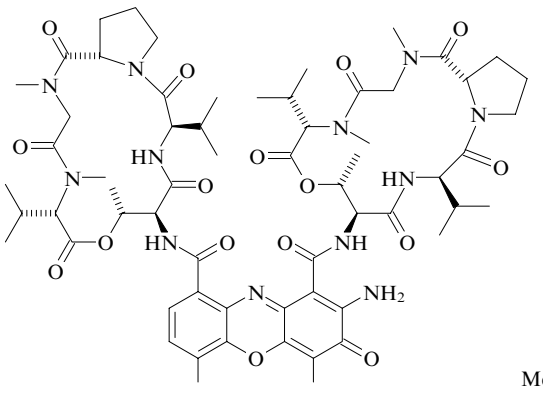
5. AT-Specific DNA minor groove-binding ligands as topoisomerase I and II inhibitors

Yet another group of topoisomerase inhibitors — DNA minor groove-binding ligands — is worthy of mention. These ligands can arrest the cell cycle (DNA strand breakage followed by repair) mediated by topoisomerases. In chemotherapy of cancer and viral infections, there is a tendency to replace intercalating drugs and alkylating agents causing non-selective DNA damages by new compounds, which noncovalently bind to DNA and influence the replication and transcription. Hence, an important problem is to design and synthesize low-molecular-weight organic compounds, which can noncovalently and site-specifically bind to the DNA minor groove.[79]

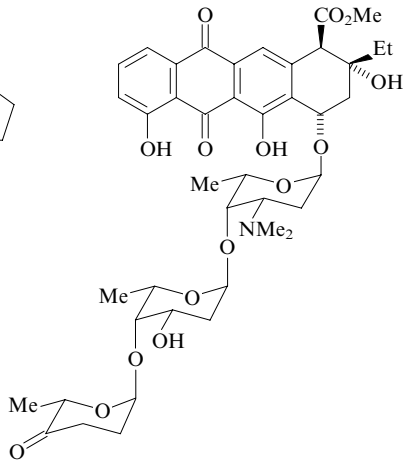
The binding to the minor groove is mediated through hydrogen bonding, as well as through electrostatic, van der Waals and hydrophobic interactions.[68,69] The inhibition of topoisomerase I by DNA minor groove-binding ligands is governed by the mechanism of their interactions with DNA.[79,80] Both neutral and positively charged compounds can bind in the minor groove of DNA. An important structural requirement for the ligand molecule is its crescent shape that closely fits the DNA minor groove. The high affinity of these compounds for DNA is also of importance. The constants and mechanism of complexation can be quantitatively estimated by spectroscopic methods (adsorption and fluorescence spectroscopy, linear circular dichroims).[81,82] Local conformational changes induced in DNA by such ligands prevent interactions between the DNA and topoisomerases. In most cases, low-molecular-weight DNA-binding ligands, unlike topoisomerase poisons, cannot stabilize DNA breaks caused by topoisomerases, but can impair the enzyme function, thus interfering with the interaction between the enzyme and DNA (suppression).[4,9] Compounds (for example, β-lapachon) that inhibit topoisomerase I activity without the formation of a ternary covalent complex[4] are mentioned above.

Hydrogen bonds formed between the ligand molecule and atoms of DNA bases play a great role in the preferential binding of minor groove-binding ligands to a particular nucleotide sequence. In most cases, these molecules exhibit selectivity to AT DNA base pairs. This is facilitated also by a substantially larger negative electrostatic potential in the AT-rich region of the minor groove compared to the GC tract.

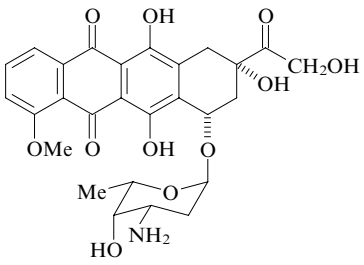
Structures 16–18



16 (actinomycin D)



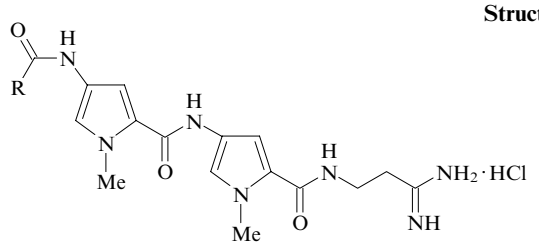
17 (aclarubicin, or aclacinomycin A)



18 (doxorubicin, or adriamycin)

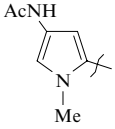
Minor groove-binding ligands that inhibit topoisomerase I — netropsin (**19a**) and distamicyn A (**19b**) — are AT-specific oligopeptide-type pyrrolecarboxamide antibiotics.[68] In solution, netropsin and distamicyn A adopt a crescent shape isogeometric to the inner space of the minor groove of the DNA double strand. Both compounds bind to the B-form DNA with high binding constants. The binding of netropsin to A- or Z-forms of DNA induces the transformation of the duplex to the B-form.[83] According to the model of the complexes of netropsin and distamicyn A with DNA proposed by Zasedatelev *et al.*,[84] the distamicyn A or netropsin molecule resides in the minor groove of DNA and covers five base pairs by hydrogen bonding between the amide nitrogen atoms of the ligand and the N(3) atoms of adenines and(or) the O(2) atoms of thymines oriented toward the minor groove. The AT specificity of the binding of these compounds can be attributed to favourable hydrogen bonding between the ligand molecule and DNA, as well as to the absence of 2-amino groups of guanine in AT-containing segments creating steric hindrance to the ligand binding. The negative electrical potential in the DNA minor groove is also energetically favourable for the interaction with the ligand bearing a positive charge.[83] Synthetic dimers of netropsin (bis-netropsins) are more effective topoisomerase I inhibitors.[85,86] It should be noted that netropsin conjugated with camptothecin proved to be a very effective topoisomerase I inhibitor and it does not cause the retardation of cell proliferation.[87]

Structures 19



19a,**b**

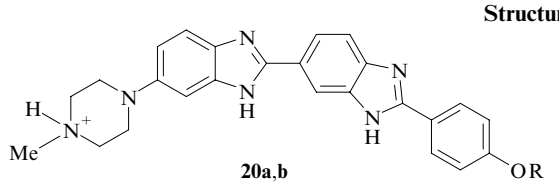
R = $HCl \cdot H_2NC(=NH)NHCH_2$ (**a**, netropsin),



(**b**, distamicyn A)

AT-Specific minor groove binders containing the bis(benzimidazole) moiety [Hoechst 33258 (**20a**) and Hoechst 33342 (**20b**)] mediate the formation of long-lived DNA–enzyme complexes in the vicinity of the DNA-binding sites of these compounds and inhibit the topoisomerase I and II activity.[88] A series of derivatives of the compound Hoechst 33342 containing three benzimidazole rings were synthesized with the aim of increasing the inhibitory activity and the antitumour effect. The inhibitory activity of these

Structures 20



20a,**b**

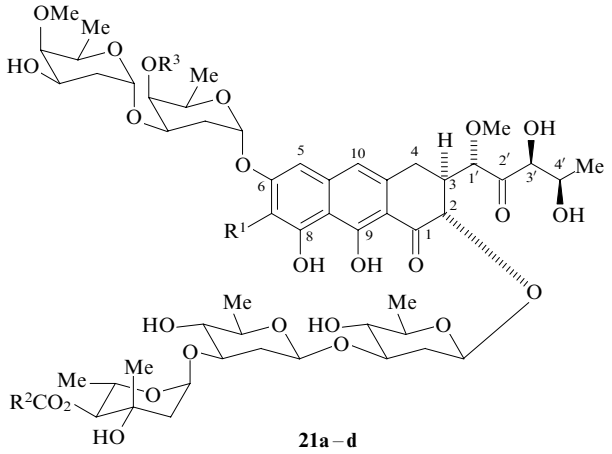
R = H (**a**, Hoechst 33258), Et (**b**, Hoechst 33342)

derivatives against topoisomerase I is higher than that of compounds **20a**,**b**.[89]

6. Other classes of topoisomerase inhibitors

The binding of bis(benzimidazole) and other AT-specific minor groove binders to AT-rich segments in the DNA minor groove limits the use of these compounds as inhibitors because it is impossible to block the enzymes in GC-rich segments. The latter are present in regulatory gene regions, the products of which are essential for the maintenance of malignant phenotypes. It was long believed that only AT-specific minor groove binders can increase the lifetime of the covalent DNA–topoisomerase complex thus interfering with DNA repair.[10] The effect of GC-specific compounds was not discussed. Bearing this in mind, compounds mimicking the topoisomerase activity by interacting with GC-rich segments of DNA are searched for. The aureoloic acid analogues olivomycins 1 and 2 (**21a**,**b**), as well as chromomycins **21c**,**d** and mithramycin, which also belong to this group of imhibitors, are GC-ligands.

Structures 21



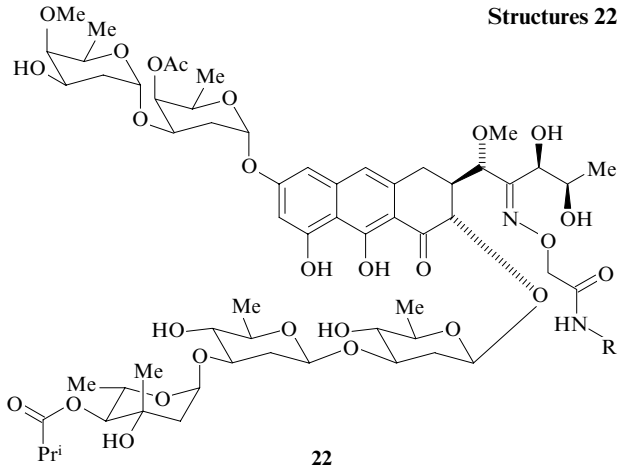
21a–d

R^1 = H, R^2 = Pr^i: R^3 = Ac (**a**, olivomycin 1), H (**b**, olivomycin 2);
R^1 = Me, R^3 = Ac: R^2 = Pr^i (**c**, chromomycin A_2),
Me (**d**, chromomycin A_3)

These compounds were shown to be capable of suppressing topoisomerase I activity.[90] Tevyashova *et al.*[91] synthesized new derivatives of olivomycin 1 (**22**) by the modification of the 2′-keto group of its side chain. These compounds proved to be topoisomerase I inhibitors. It should be emphasized that olivomycin 1 is considered as the gene transcription inhibitor.[92]

As opposed to anthracycline antibiotics serving as intercalators, the mechanism of action of GC-specific analogues of aureoloic acid is non-intercalation. The mechanism of complexation involves the interaction between the compounds and the GC base pairs in the DNA minor groove complexed with divalent metal cations (Mg^{2+}, Mn^{2+}, Fe^{2+}).[90,93] This complexation damages DNA and impairs matrix syntheses, in particular, the transcription.[94,95] Both carbohydrate chains (disaccharide and trisaccharide) are essential for biological properties of aureoloic acid, and two acyl groups in the carbohydrate moieties are important for olivomycin and chromomycin derivatives.[96,97] The aglycon of olivomycin (**23**, olivin), unlike highly toxic olivomycins and chromomycins, does

Structures 22

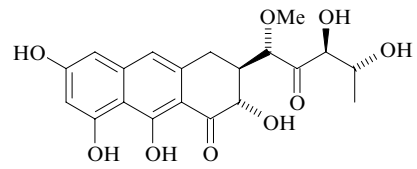


22

R = H, $(CH_2)_2OH$, Bu^t, $CMe(CH_2OH)_2$, 2-adamantyl

not bind to DNA, does not cause the cell death [95] and does not inhibit topoisomerase I.[98]

Structure 23



23 (olivin)

Compounds that predominantly bind to AT- or GC-rich segments of DNA can change the activity not only of topoisomerases but also of other DNA-modifying enzymes due to the formation of stable complexes with DNA and local conformational distortions of the duplex. These issues, as well as the cytotoxicity of the above-mentioned compounds, were analyzed in detail by Russian research teams.[83–87, 95] Such DNA-binding ligands are unlikely to be widely used as drug candidates due to the absence of enzyme specificity of these compounds. Requirements currently imposed by pharmaceutical companies on the drug design include high specificity to the inhibited target. The above-considered chemical classes play an important role as experimental tools for investigating structure–activity relationships and mechanisms of inhibition of topoisomerases by different compounds.

IV. Methodological approaches to the determination of the inhibition of topoisomerases

An analysis of changes in the enzyme activity in a cell-free system is an important stage in the design of topoisomerase inhibitors. However, the accuracy of modelling of intracellular processes in *in vitro* experiments is rather low. Not any compound that inhibits the topoisomerase activity in a cell-free system penetrates into the cell and subsequently into the cell nucleus. The assay used for the examination of the ability of compounds to inhibit topoisomerases I or II (topoisomerase I/II assay) is based on the difference in the electrophoretic mobility of DNA molecules having different conformations (topoisomers) (Fig. 1).[15, 99] Topoisomerase I converts supercoiled DNA into a number of relaxed DNA topoisomers, each differing from adjacent topoisomers by ±1 link. More tightly wound DNA molecules migrate more rapidly. In the presence of tested compounds, the formation of relaxed DNA topoisomers is retarded up to the complete cessation of plasmid relaxation. Data on interactions between the tested compound and DNA are required for the detailed investigation of the targeted action. Besides, it is necessary to confirm the existence of long-lived DNA breaks in order to judge the formation of ternary covalent complexes. These tasks can be accomplished using a wide range of methods, such as X-ray crystallography, spectroscopic analysis, radioactive labelling of DNA breaks, the quantitative detection of the covalent complex in the presence of K^+ and sodium dodecyl sulfate and so on.[3, 35, 100]

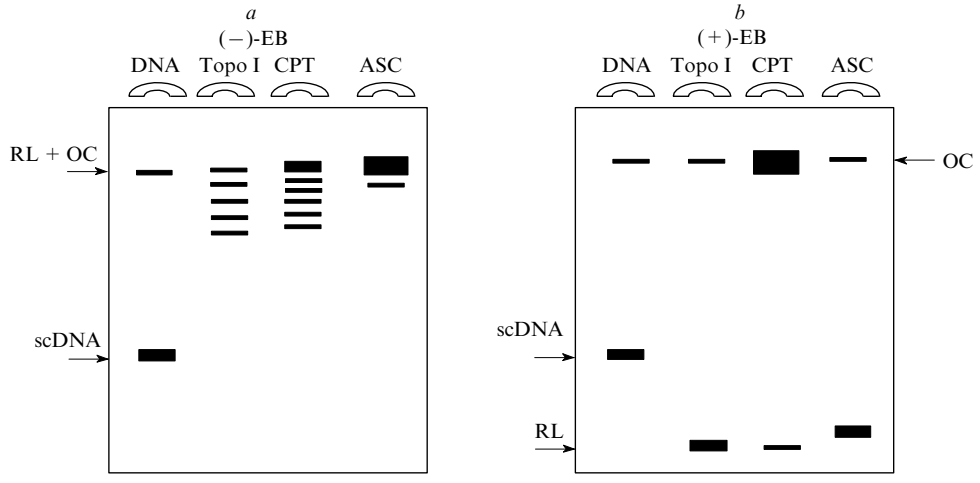


Figure 1. Influence of ethidium bromide on the separation of DNA topoisomers by agarose gel electrophoresis.[15]
The native supercoiled plasmid pKMp27 (lane scDNA) was incubated with topoisomerase I in the absence (Topo I) and in the presence of camptothecin (CPT) or ascididemin (ASC).
(*a*) The gel was stained with an ethidium bromide solution after electrophoresis; (*b*) ethidium bromide was added to the gel before electrophoresis. scDNA is the supercoiled plasmid DNA, OC is the open circular form of DNA (the DNA form with a single-strand break), RL is the relaxed plasmid DNA.

Assays like those described above can provide preliminary (though necessary) information on the presence or the absence of inhibitory activity of the compound. This method can be used to distinguish between intercalation-induced nonspecific effects and specific effects (poisoning of topoisomerase I). A strong intercalator inserts between bases of the duplex and causes substantial changes in the DNA structure thus suppressing the relaxation. The migration rate of supercoiled DNA in gel containing ethidium bromide (EB) decreases with increasing concentration of such intercalators, which can be attributed to a decrease in the number of links in the supercoiled plasmid due to intercalation properties of these compounds. This feature confirms the nonspecific character of inhibition of topoisomerase I by intercalators. The electrophoretic mobility of the open form of DNA remains virtually unchanged because of the weaker intercalation into open circles of DNA.[15] Thus, in the EB-containing gel the relaxed form of DNA (closed circular form of DNA) migrates faster than the supercoiled plasmid due to inhibition of the DNA unwinding caused by the interaction between DNA and the strong DNA intercalator (ethidium bromide). The migration of the open circular form of DNA (in the case of topoisomerase I) or the linear form (in the case of topoisomerase II) in gel is retarded compared to the above-mentioned forms. Therefore, all three forms of DNA are separated in gel and, consequently, can be identified.

The difference in the mechanisms of inhibition of topoisomerase I can be shown by considering the effect of the specific inhibitor camptothecin and the nonspecific inhibitor ascididemin on the relaxation of the plasmid. The ability of the latter to intercalate is a decisive factor for the suppression of the relaxation of the plasmid DNA by topoisomerase I. The specific inhibitor camptothecin prevents DNA repair, thus also inhibiting this enzyme but in another way, by forming a ternary covalent complex. For migration in gel in the absence of EB, the effect of camptothecin is comparable to that of ascididemin (Fig. 1 *a*). The electrophoresis in the EB-containing gel (Fig. 1 *b*) shows that ascididemin is not a potential poison of topoisomerase I. As opposed to camptothecin, the band corresponding to the open form of DNA is only slightly more intense in the presence of the DNA intercalator ascididemin. Actually, ascididemin weakly stabilizes the covalent DNA–topoisomerase I complex.[15]

V. Modelling and computational design of topoisomerase inhibitors

The development of methods for the analysis of conformational dynamic properties of macromolecules and their complexes, as well as investigations of models of processes in the living cell, are of great help in the design of novel drugs.[101] Computational modelling methods based on special simulation algorithms for biological processes, their construction and visualization are employed for this purpose.[102] Mathematical modelling becomes an important tool of an integrated approach to elucidating mechanisms of complex processes, in particular, to interpreting experimental data and predicting the behaviour of biomacromolecules under specified conditions. The three-dimensional structures of targets are determined by X-ray crystallography and NMR spectroscopy. Partial charge calculations for atoms of interacting models and the search for minimum-energy conformations necessary for modelling intermolecular interactions are carried out by quantum chemical and molecular dynamics methods. The interactions are simulated and their energies are estimated by molecular docking and molecular mechanics. The structure–activity relationships are studied by QSAR analyses. Modern computational techniques allow one to take into account the effect of the environment on intermolecular interactions, such as the presence of the solvent, its ionic strength and the role of the cofactor. Along with the structural modelling on a local scale dealing only with intermolecular interactions, the kinetics of interactions between molecules is also simulated.

The most considerable progress was achieved in the molecular simulation of proteins and their interactions with low-molecular-weight chemical compounds. At the present time, calculation of such interactions is an essential and necessary step in the drug design, in which particular proteins serve as targets. The crystal structures of hundreds of proteins and their complexes with activators or inhibitors of cytoplasmic and membrane proteins were determined, and numerous computational algorithms were developed.[103–107] Complexes of DNA and DNA-modifying enzymes [for example, poly(ADP-ribose) polymerases] with chemical ligands were studied in less detail, although this field of computational chemistry is in active progress.[82, 108, 109]

Examples of the structural modelling in order to search for new topoisomerase inhibitors are given below. Interactions between camptothecin derivatives and topoisomerase I were analyzed using the Discovery studio 2.0 software (Accelrys, USA) and the docking procedure, which takes into account the conformational lability of the ligand and allows for the flexibility of the target at the binding site.[110, 111] The model of complexes between camptothecin and topoisomerase I was obtained[114] using the Auto-Dock 3.0.5 software[112] and the Lamarckian genetic algorithm.[113] Models of interactions of eicosapentaenoic acid and liriodenine derivatives with topoisomerases I and II were obtained with the use of the Auto-Dock 4 software employing the genetic algorithm and simulated annealing.[115–118] The docking was performed also using the Sybyl molecular graphics program (Tripos Inc., USA) for the modelling of protoberberines as topoisomerase I inhibitors.[119] The possibility of the inhibition of topoisomerase I by acridine derivatives was predicted using the ICM Biased Probability Monte Carlo method[120] implementing flexible ligand docking.[121] In some cases, the stability of complexes calculated using the docking procedure was verified by the molecular dynamics. For instance, Tesauro *et al.*[122] modelled erybraedin C (the compound from the plant *Bituminaria bituminosa*) as a topoisomerase I inhibitor. The docking was carried out based on the Lamarckian genetic algorithm using the Auto-Dock 4 software, and the results were analyzed using the Gromacs 3.3.3 package.[123] This package and molecular dynamics methods were used to simulate the interaction between topotecan and topoisomerase I.[124] It was found that topotecan interacts with the amino acid residues Lys532 and Arg364 of topoisomerase I involved in the binding to the minor groove of DNA. To elucidate the mechanism of inhibition of topoisomerase I by compounds of the benzo[*c*]phenanthridine class, Clark and co-workers[125, 126] employed the controlled molecular dynamics. As an example of the use of QSAR tools, we

would mention calculations, which revealed topoisomerase I inhibitors that bind to the minor groove of DNA.[127] Benzimidazoles, bis(benzimidazoles) and tris(benzimidazoles) were analyzed and a model of the inhibition for numerous minor-groove ligands of these chemical classes was described.

VI. Conclusion

The design of modern target-directed drugs relies on two complementary approaches: the chemical library screening (and heuristic search to discover active compounds) and the rational design based on the knowledge of the structures of target proteins and low-molecular-weight ligands. The above analysis of the published data shows that both approaches are suitable for the search for new topoisomerase inhibitors and optimization of the known inhibitors. However, the aim is still not reached. Problems common to the design of antitumour agents remain to be solved: the targeted delivery to cancer cell with minimal damage to surrounding healthy cells and the controlled distribution in the body (to reduce systemic toxicity); the identification of tumours, the therapeutic treatment of which requires the inhibition of topoisomerases; overcoming multifacotrial drug resistance in cancer cells and so on. The progress in the solution of these problems is beyond the scope of medicinal chemistry, which is the subject of the present review. Nevertheless, the importance of the 'chemical' step in the drug design cannot be overstated. New molecules draw attention from biologists, pharmacologists and clinicians.

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